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STIC-ILL

450039

From: Saucier, Sandy
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To: STIC-ILL
Subject: FW:

Sandra Saucier
AU 1651

11B01

for 09/828413

10807895

L35 ANSWER 2 OF 2 MEDLINE
AN 2001029686 MEDLINE
DN 20529031 PubMed ID: 11074924
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Government and Medical Education

TO THE EDITOR: Dr. Kefalides did a nice job summarizing some of the issues surrounding the role of government in medical education (1).

However, I was dismayed to read a section quoting Wanda Wallis from the Medical Board of California. She states that she was unable to document the compliance of Loyola Stritch School of Medicine with the family medicine clerkship requirement. I am happy to report that our family medicine clerkship, required at the school, has been the highest-rated clerkship since 1995. Family medicine is the second most frequently chosen specialty of our graduates (internal medicine is first), and many of our graduates have gone to California for their residency training. Our students rotate through a variety of sites, including community hospitals such as West Suburban Hospital in Oak Park, Illinois. Family medicine is a large part of the curriculum at Loyola Stritch, and the article leads one to think otherwise. Specifically, I am concerned that anyone reading the article would conclude that the school does not have a required family medicine clerkship. This could not be further from the truth.

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Reference

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A Question of Ethics

TO THE EDITOR: Certification by the American Board of Internal Medicine includes an evaluation of "moral and ethical behavior in the clinical setting" for the medical resident.

Some residents commit to a subspecialty training program, then fail to follow through, leaving the program high and dry for a fellow. This occurs only too often when a resident is in an exchange visa program, which stipulates that after fellowship the physician must leave the United States for at least 2 years. However, if a graduate of a residency program practices in a medically underserved area in the United States after residency, he or she may obtain a waiver for this waiting period.

Sadly, I have seen residents look me straight in the eye and assure me that they are committed to doing a fellowship. Then, after contracting for a position, the resident will clandestinely seek a practice in an underserved area. If such a position is obtained, the resident will withdraw from the specialty program. When the resident is confronted, I have received such replies as "I was advised not to tell you the truth, because that might prevent me from staying in this country." It is a clear "end justifies the means" message that is repeatedly offered.

The preceding may be just one narrow example of an "ethical lapse." We are still proud of the broad cultural diversity of our program's residents and faculty. If we ever hope to improve our residents' behavior, we need first to look at ourselves. Will physicians order unnecessary tests to enhance their income? Do physicians fabricate patient information to justify insurance reimbursement for a patient's needed care? It seems we need better ways to guide our residents through postresidency placement and better ethical measures for our collective behavior.

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Successful Treatment of Hepatitis C in Sickle-Cell Disease

TO THE EDITOR: Patients with sickle-cell disease are at high risk for hepatitis C virus (HCV) infection; HCV seropositivity rates in this group are as high as 30% (1). In the U.S. population, 1.8% of patients with this disease are infected with HCV (2). Cirrhosis caused by HCV may supervene as an important cause of death as patients with sickle-cell disease live longer. Indeed, liver transplantation for HCV-related cirrhosis has been performed in patients with sickle-cell disease (3). Although patients with sickle-cell disease commonly survive to their sixth decade (4, 5), treatment of HCV infection in these patients has not been reported.

We evaluated a 35-year-old woman with HCV acquired from blood transfusions that had been done for hemoglobin SS sickle-cell disease. In July 1998, the patient had an HCV RNA level of 101 053 copies/mL, with mildly elevated aminotransferase levels. The albumin level was 606 μmol/L, the leukocyte count was 12.9×10^9 cells/L, the hemoglobin level was 98 g/L, the hematocrit was 0.28, the platelet count was 361×10^9/L, and the ferritin level was 1382 μg/L. Liver biopsy disclosed portal lymphocyte infiltration and notable portal fibrosis consistent with active HCV infection. Hemosiderosis was also noted.

We instituted therapy with hydroxyurea, 500 mg orally twice daily, resulting in a fetal hemoglobin level of 0.198. We then began therapy with interferon-α2b, 3 million U subcutaneously three times weekly, and ribavirin, 1 g orally per day. Except for mild constitutional symptoms, the patient's interferon–ribavirin treatment was notable for stable hematocrit and complete absence of painful episodes. After 20 days of treatment, liver enzyme levels were normal and HCV RNA was undetectable. On day 127 of treatment, HCV RNA remained undetectable. The patient completed 26 weeks of treatment without complication.

We subsequently treated HCV infection in a second patient with sickle-cell disease. This 61-year-old woman had an initial HCV RNA level of 292 000 copies/mL. After 3 months of interferon–

ribavirin treatment, HCV RNA became undetectable. In the absence of painful episodes or worsened anemia, she is continuing therapy. Our experience suggests that antiviral HCV treatment has a broader role in the care of patients with sickle-cell disease than was previously supposed.

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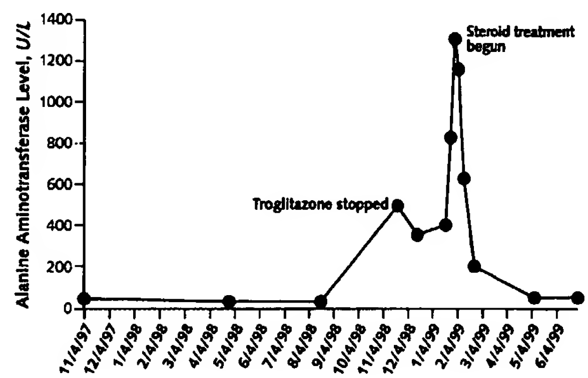
Troglitazone-Associated Hepatotoxicity Treated Successfully with Steroids

TO THE EDITOR: A 59-year-old diabetic man was referred to a transplantation clinic with progressive liver dysfunction. He had begun feeling increasingly fatigued 5 months earlier. Liver enzyme levels were elevated (alanine aminotransferase [ALT] level, 8350 nkat/L; aspartate aminotransferase [AST] level, 4.98 μkat/L), and troglitazone and simvastatin therapies were stopped. During the next 8 weeks, aminotransferase levels continued to increase to 21 667 nkat/L, and the bilirubin level increased from normal to 308 μmol/L (18 mg/dL) (Figure).

The patient had initiated troglitazone therapy 1 year before presentation. At baseline and periodic checks, liver enzyme levels were within normal limits. Other medications included ramipril, diltiazem, furosemide, insulin, and glyburide. Alcohol intake was insignificant. On evaluation at our center, total bilirubin level was 444 μmol/L (26.0 mg/dL), alkaline phosphatase level was 7.36 μkat/L, ALT level was 10 583 nkat/L, and AST level was 5.26 μkat/L. Prothrombin time was normal. Laboratory investigation included negative results on viral serologic testing, normal immunoglobulin levels, and mild positivity for antinuclear and anti–smooth-muscle antibodies (both at a titer of 1:80). Ultrasonography and computed tomography were unremarkable. Liver biopsy showed submassive necrosis. The overall presentation was considered consistent with troglitazone-induced hepatotoxicity.

Figure. **Relationship of alanine aminotransferase level to cessation of troglitazone therapy and initiation of steroid therapy.**



To convert alanine aminotransferase values to nkat/L, divide by 16.667.

The patient was prescribed prednisone, 20 mg twice daily. Two weeks after initiation of steroid therapy, total bilirubin, AST, and ALT levels decreased to 128 μmol/L (7.5 mg/dL), 1.62 μkat/L, and 3450 nkat/L, respectively. At 5-month follow-up, the patient was no longer receiving steroids and had normal liver enzyme levels.

Steroids may be appropriate treatment of troglitazone-associated hepatotoxicity. Because many of these patients have progressive liver dysfunction that may lead to transplantation and death (1–5), the discovery of an easily administered medical therapy would have a substantial effect on patients with this disorder.

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STIC-ILL

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Am rican Journal of Hemat logy 32:104–111 (1989)

Effect of Hydroxyurea on the Rheological Properties of Sickle Erythrocytes In Vivo

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We have monitored the rheological effects of hydroxyurea (HU) on erythrocytes obtained from two patients with severe sickle cell anemia who were enrolled in a therapeutic trial of this drug. Erythrocyte membrane stability and whole cell and membrane deformability of red cells from treated and untreated patients and normal controls were determined in room air using an ektacytometer—a laser viscodiffractometer. The percentage of dense cells was quantitated by centrifugation on a discontinuous Stractan density gradient. F reticulocytes (FR), absolute F reticulocytes (AFR), and F cells (FC) were determined by single-cell radial immunoigic assays. After 1 year of treatment with HU, there was a significant increase in the level of hemoglobin (Hb) F, FR, AFR, and FC. The degree of anemia remained the same, but there was significant increase in the mean cell volume (MCV) and a significant decrease in the mean corpuscular Hb concentration (MCHC). Whole cell deformability improved by twofold, but membrane stability remained within normal limits. The hydration status of sickle erythrocytes improved as was indicated by a change toward normal in gradient osmotic ektacytometry, an increase in RBC K^+ content, a decrease in percent of dense cells, and a decrease in the MCHC. The data indicate that, in addition to its effect on the production of Hb F, HU has a salutary effect on whole cell deformability and on the hydration status of sickle erythrocytes. Determination of the rheological properties of erythrocytes may be of value in monitoring the response to HU.

Key words: sickle cell anemia, red cell deformability, erythrocyte deformability

INTRODUCTION

Long-term treatment of adult patients suffering from severe sickle cell anemia with hydroxyurea (HU) results in significant elevation of hemoglobin (Hb) F [1]. Because the latter is known to inhibit the polymerization of Hb S [2,3], HU seems to be a potential candidate for the treatment of sickle cell anemia in an effort to ameliorate the clinical manifestations of this disease. Other effects of HU include macrocytosis and increase in the mean corpuscular hemoglobin (MCH) [1,4,5]. Moreover, one report [5] indicated that red blood cells (RBC) from a patient with psoriasis treated with HU showed the presence of a significant population of hypodense, presumably overhydrated, cells by Percoll-Stractan density gradient centrifugation. The approximate mean corpuscular Hb concentration (MCHC) of this cohort of hypodense cells was decreased to 28 g/dl. Since the MCHC is an important determinant of erythrocyte deformability [6], we thought that HU may have an effect on the rheological properties of the RBC of patients with sickle cell anemia treated with this drug. To test this hypothesis we monitored the rheological effects of HU on RBC from two patients with severe sickle cell anemia taking this drug. The data show that HU improves both the deformability and the hydration status of sickle erythrocytes in vivo.

MATERIALS AND METHODS

Patients

Two patients (SC and FT) with homozygous sickle cell anemia refractory to conventional therapy were

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started on HU. They were participants in an open therapeutic clinical trial of this drug, the details of which will be described elsewhere. The initial daily dose was 1,000 mg/day. Within 2–3 months, this was increased by 500 mg 3 days/week. Blood samples were obtained from both patients before starting HU when they were in their asymptomatic steady state and on a weekly basis after starting the drug. Both patients required occasional blood transfusion. The data presented in this study were those determined at least 4 months after the last transfusion. One of the patients (FT) is known to have multiple rare alloantibodies secondary to blood transfusion and this aspect of his disease was previously described [7].

Hematologic Data

Before treatment with HU, the RBC indices were determined by the semi-manual method, i.e., the hematocrit (Hct) was measured by centrifugation, the Hb by the cyanmethhemoglobin method, and the RBC were counted on a Coulter model F cell counter (Coulter Electronics, Hialeah, FL). After treatment with HU, RBC indices were determined by the Technicon H.1 System, which also gives accurate values of the MCHC. These two methods of estimating red cell indices were reported to correlate well with each other [8]. The percentage of Hb F in hemolysates was measured by alkali denaturation [9]. The proportions of Hb F cells (RBC that contain Hb F) and F reticulocytes (reticulocytes that contain Hb F) were assayed by single cell radial immunologic technique by previously described methods [10]. RBC sodium and potassium concentrations were determined by flame photometry of RBC aliquots washed in ice-cold, isotonic Tris-buffered $MgCl_2$ solution (10 mmol/liter Tris HCl, pH 7.4).

RBC survival was determined by a modification of the standard ^{51}Cr technique [11] as described previously [12]. Thirty milliliters of autologous cells were labeled with 100 μCi of ^{51}Cr. Fifteen milliliters of labeled RBC (Hct 45%) were injected, and subsequent samples were taken for survival measurements. The 10 min sample was considered the baseline to which the other samples were compared, and a percentage of survival was calculated [7,11].

Whole Cell Deformability Measurement

The ektacytometer, a viscodiffractometer, designed and previously described by Bessis and Mohandas and colleagues [6,13,14], was used to measure whole cell deformability in room air as a continuous function of the suspending medium osmolality at a constant applied shear stress of 170 dynes/cm^2 (osmotic gradient ektacytometry). For these studies, the deformability index (DI) of RBC was continuously recorded as the suspending medium osmolality was increased from 50 to 500 mosmol/kg as previously described [15].

Preparati n f Resealed Erythr cyte Membranes

Resealed membranes for deformability and stability measurements were prepared by a procedure described by Johnson [16]. Erythrocytes from normal blood donors and patients were drawn into acid citrate dextrose tubes and washed three times in 5 mM Tris and 140 mM NaCl, pH 7.4. Washed cells were lysed in 40 vol of ice-cold 7 mM NaCl and 5 mM Tris, pH 7.4, and the ghosts were washed free of Hb in the same lysing buffer. The membranes were pelleted by centrifugation; resuspended in 10 vol of 5 mM Tris and 140 mM NaCl, pH 7.4; and incubated 60 min at 37°C for resealing. The resealed membranes were pelleted by centrifugation and prepared for ektacytometry as described below.

Membrane Deformability Measurements

Resealed membranes, prepared as described above, were suspended in 7.0 ml of Stractan II (Champion International, Tacoma, WA) solution with a viscosity of 22 cp (290 mosmol, pH 7.4) and examined by ektacytometry. Suspended, resealed membranes were exposed to an increasing shear stress (0–200 dynes/cm^2), and the change in their laser diffraction pattern from circle to ellipse was measured. For resealed membranes, the shear stress required to obtain a defined value of DI is determined by the property of membrane deformability without contribution from either internal viscosity or cell geometry [17,18]. There is a correlation between changes in deformability measured by this technique and those measured using the micropipette [19]. Analysis of the DI curve generated by the ektacytometer thus provides a measure of membrane deformability [17,18].

Membrane Stability Measurements

For measurement of membrane stability, 100 μl of resealed membranes was suspended in 7.0 ml dextran solution (molecular weight 40,000; 35 g/dl weight/vol) having a viscosity of 97.5 ± 2.5 cp. The pH was maintained at 7.4 with 10 mM phosphate buffer, and the osmolality was adjusted to 290 ± 5 mosmol/kg with NaCl. Samples were brought to the maximum shear stress within 20 sec and maintained at that level until the DI fell to a constant plateau. The applied shear stress was 750 dynes/cm^2 as calculated from the solution viscosity and the shear rate of 760 sec^{-1} provided by the ektacytometer. Under this stress, the membranes progressively fragmented, generating undeformable spherical fragments [20].

TABLE I. Effect f Hydroxyurea n Hemat logical Parameters After 1 Y ar of Treatment

	Patient SC		Patient FT	
	Before HU	After HU[a]	Before HU	After HU[a]
Hb (g%)	7.0	6.1–8.5	8.6	6.0–9.0
MCV (fl)	91	120–135	82	111–122
MCHC (%)	36.0	29.2–32.5	34.5	29.7–31.7
Reticulocytes (%)	14.6	4.9–14.2	7.4	3.5–9.4
Hb F (%)	0.7	15.9–30.6	2.3	14.6–20.4
F Retic (%)	1.3	15.3–34.7	3.7	14.0–24.7
F Cells (%)	5.5	65.3–80.4	18.7	66.9–71.5
Absolute F Retic. ($10^3/\mu l$)	2.3	20.7–97.6	7.8	17.3–40.9
Dense cells (%)	12.9	<1.0	7.0	<1.0
$T_{1/2}$ (days)	3.5	16.0	13.0	23.0

[a]Values after HU are those determined from day 363 to day 505 of drug therapy.
Therapy on these days was 1,500 mg on Mondays, Wednesday, and Fridays and 1,000 mg on Tuesdays, Thursdays, Saturdays, and Sundays.

Separation of RBC by Density

Subpopulations of erythrocytes of uniformly defined densities were isolated on discontinuous Stractan II density gradients by centrifugation of normal and sickle RBC [21]. The gradients covered a density range from 1.065 to 1.139 g/ml in increments of 0.004 g/ml.

Determination of Dense Erythrocytes

The proportion of dense erythrocytes was measured by cyanmethemoglobin determination [22] of the fraction of Hb found in a dense (d = 1.110 g/ml) cushion of Stractan II in a 100 μl microhematocrit tube (Corning Glass Works, Corning, NY) after a 20 min centrifugation in a microhematocrit centrifuge as previously described [23].

Determination of α-Globin Genotypes

α-Globin genotypes were determined by Southern blot hybridization [24] of genomic DNA extracted from peripheral blood leukocytes. The restriction endonucleases used were Bam H1 and Bgl II, and the probe was Bam H1-linearized α-cDNA JW101 plasmid [25], which was labeled with ^{32}P by nick translation.

RESULTS

α-Globin Genotypes

Patient SC had four α genes (αα/αα genotype), and patient FT had 3α genes (−α/αα genotype). This difference in α genotypes between the two patients explains the relatively low baseline values of the MCV and the MCHC of the RBC of patient FT (Table I).

Effect of Hydroxyurea on Hematological Parameters

Table I shows the effect of hydroxyurea on the hematological parameters studied. Typical and representative data for each patient before starting hydroxyurea are listed. The range of the values for each parameter 1 year after starting HU is indicated. HU had little effect on the Hb and reticulocyte values. Besides increasing the level of Hb F, F cells, and F reticulocytes, HU increased the MCV and decreased the MCHC as measured by the Technicon H.1 System and the percent of dense cells. Moreover, HU improved the RBC survival in both patients.

Effect of HU on RBC Deformability

The effect of HU on the deformability profile of erythrocytes from both patients is shown in Figure 1. These were determined on days 343 and 335 of therapy for patients SC and FT, respectively. The drug improved whole cell deformability at all levels of osmolality. The maximum deformability increased to values above normal, indicating increased surface area to volume ratio [15,21]. The descending arm of the curve shifted to higher osmolality levels, indicating decrease in intracellular viscosity secondary to decreased Hb concentration [15,21]. The temporal changes in MCV, reticulocyte count, Hb F, percent F cells, and whole RBC deformability after treatment with HU are shown in Table II. Before treatment with HU, the DIs at physiologic tonicity of 290 mosmol/kg were 24% and 36% of control for patient SC and FT, respectively. After 7 months of treatment, the DI increased to about 80% of normal values in both patients and remained at that level thereafter (Table II). Noteworthy in Table II is that the DI improved within 2 months of therapy, although Hb F was low and the MCV was within the upper normal range. After 7–8 months of therapy (Table II, days 230 and 259), the MCV was in the 120 fl range, Hb F was 12–14% and the DI was over 80% of control values at isotonic conditions.

Effect of HU n RBC Hydration

To elucidate further the hydration status of RBC from both treated patients, we determined the cation content

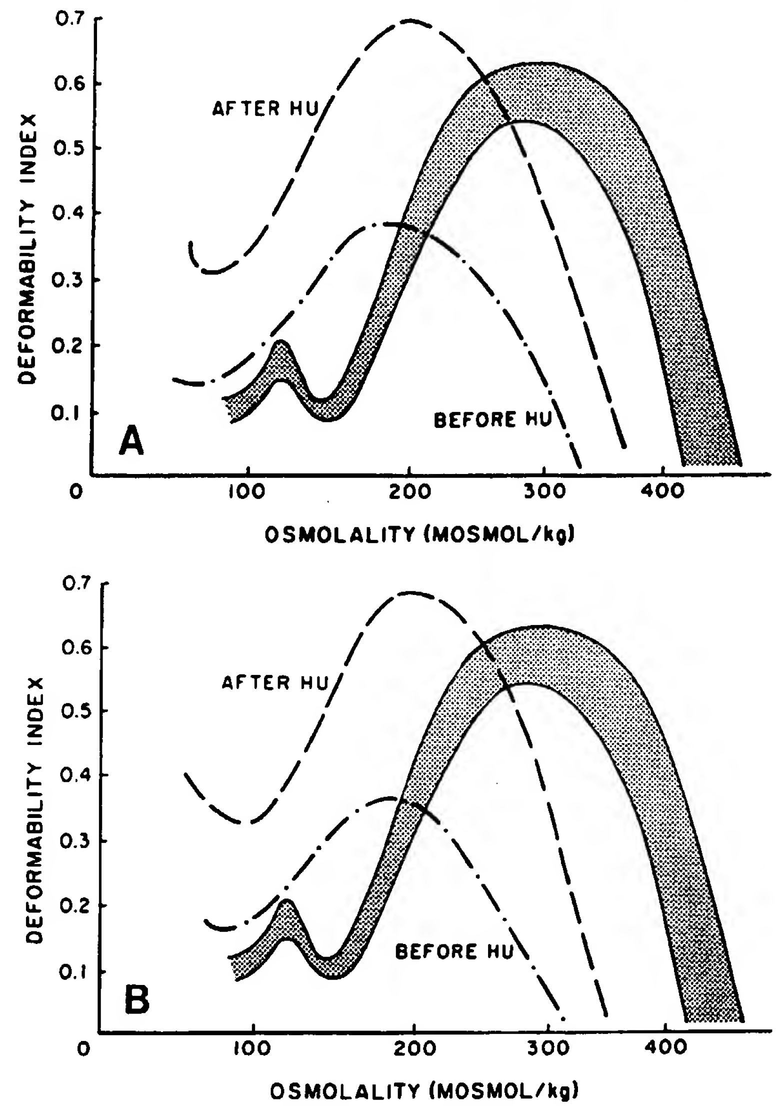

Fig. 1. Osmotic deformability profile before and after treatment with hydroxyurea (HU). The shaded area represents the range for normal controls. A: Patient SC. Hematologic values for the profile on day 343 of therapy were as follows: MCV= 129 fl,re-tic.= 10.4%, Hb F = 23.3%, and F cells = 83.8%. B: Patient FT. Hematologic values for the profile n day 335 of therapy were as follows: MCV = 114 fl, retic. = 10.6%, Hb F = 16.3%, and F cells = 71.5%.

and RBC distribution by density before and after treatment with HU. Table III summarizes the RBC cation content before and after treatment. RBC Na decreased significantly ($P < 0.001$) in patient SC, and K increased ($P < 0.001$) in both patients. There was also a net total RBC cation increase ($P < 0.001$) in both patients, indicating improved cellular hydration. Further evidence of improved hydration is shown in Figure 2A, which demonstrates very few high-density sickle erythrocytes on discontinuous Stractan density gradient. Both patients had <1.0% of dense RBC after treatment with hydroxyurea compared with 12.9% and 7.0% before therapy (Table I). Unfortunately, we did not take a picture of the Stractan gradients before starting HU. Figure 2B, however, shows the Stractan density gradients of RBC of other patients with sickle cell anemia in the steady state and of comparable clinical picture and α-gene number. It is obvious that these patients do have a remarkably higher number of dense RBC than patients SC and FT.

Effect of HU on Membrane Deformability and Stability

To determine if the improvement seen in whole cell deformability after HU is due solely to decreased intracellular viscosity, we determined the deformability of isolated and resealed RBC membranes after therapy with

TABLE II. Temporal Effect of Hydroxyurea on RBC Parameters, Hb F Production, and RBC Deformability

Patient SC[a]						Patient FT[b]					
Days of Rx	MCV	Retic.	Hb F (%)	F cells (%)	DI[c]	Days of Rx	MCV	Retic.	Hb F (%)	F cells (%)	DI[c]
28	100	11.3	0.5	4.8	—	20	90	7.6	2.3	14.8	56
57	104	12.2	1.9	18.6	43	34	91	5.4	3.0	20.6	—
77	114	10.4	—	16.0	50	66	94	8.6	—	—	63
120	96	9.5	3.9	18.9	—	69	97	16.6	—	25.4	—
213	98	7.6	5.4	24.2	—	111	113	16.8	4.2	26.6	—
245	120	15.2	12.0	50.9	—	139	100	8.8	5.8	32.3	—
259	125	13.0	13.3	—	80	181	103	6.2	8.6	40.1	103
273	126	9.9	15.3	68.0	94	195	106	7.2	10.6	51.7	82
288	132	15.6	17.8	—	88	230	114	7.2	13.3	—	94
301	127	7.9	20.8	84.0	—	237	120	6.7	14.2	63.3	—
311	127	7.9	24.2	—	97	265	115	4.9	13.1	63.5	97
337	129	10.4	22.4	—	75	335	114	10.6	16.3	71.5	76
343	131	12.9	23.3	83.8	80	373	117	9.7	—	—	85
364	135	5.8	22.2	80.4	—	385	118	8.4	16.6	67.6	—
371	128	9.4	25.2	—	88	412	111	5.8	17.9	63.8	—
407	122	7.7	19.4	—	87	440	120	6.6	14.6	63.8	84
420	126	10.5	20.8	69.2	—	454	113	6.5	12.8	—	85
434	133	14.2	16.4	—	88	468	116	6.8	18.1	70.8	—
462	120	9.4	18.6	—	97	496	114	9.5	17.8	66.9	88
505	123	13.9	20.8	77.2	93	503	117	9.9	17.2	—	81

[a]Transfused on days 87, 92, 140.
[b]Transfused on days 72, 91.
[c]DI, deformability index at 290 mosmol/kg expressed as percent of value of normal control determined in parallel.

TABLE III. Effect of Hydroxyurea on RBC Cations*

	Cations (mEq/10g Hb)		
	Na	K	Na + K
Patient SC			
Before HU (6)	0.56 ± 0.10	2.03 ± 0.12	2.59 ± 0.11
After HU (5)[a]	0.27 ± 0.02	2.88 ± 0.20	3.15 ± 0.20
Patient FT			
Before HU (12)	0.53 ± 0.08	2.65 ± 0.21	3.18 ± 0.17
After HU (6)*	0.46 ± 0.04	3.17 ± 0.13	3.63 ± 0.14

*Values are mean ± SD. Number of determinations is indicated in parentheses.
[a]Values after HU are those determined between day 301 and day 462 for SC and between days 265 and 454 for FT.

HU. This was done on days 337, 343, and 505 of therapy for patient SC and on days 335, 427, and 496 for patient FT. Figure 3 shows the membrane deformability data, with the DI expressed as a percentage of corresponding controls at a certain shear stress (100 dynes/cm^2). Resealed membranes from the treated patients require less shear stress than normal membranes to reach equivalent deformation, indicating that their RBC membranes are less rigid than normal ones. These data, however, must be interpreted with caution, since the apparent increase in membrane deformability is, at least in part, due to a greater surface area as evidenced by an increased MCV value. Red cell membranes from patients with sickle cell anemia who are not on HU did not show increased deformability. Figure 4 shows that treatment with HU had no effect on membrane stability.

DISCUSSION

Our results show that HU has interesting hemotological and rheological effects on RBC in addition to its known effects on the production of Hb F [1,10,26]. The Hb level in both patients did not change appreciably after treatment with the drug despite a significant improvement in RBC survival. This constellation of findings suggest that HU might have had a mild myelosuppressive effect on the bone marrow, which was counterbalanced by the improved erythrocyte survival in peripheral blood, thus keeping the Hb level within the same range. The MCV increased in both patients and the percent increase was similar in both cases (32–49%). The absolute value of the MCV in the patient with three α genes, however, remained about 10 units less than that of the patient with

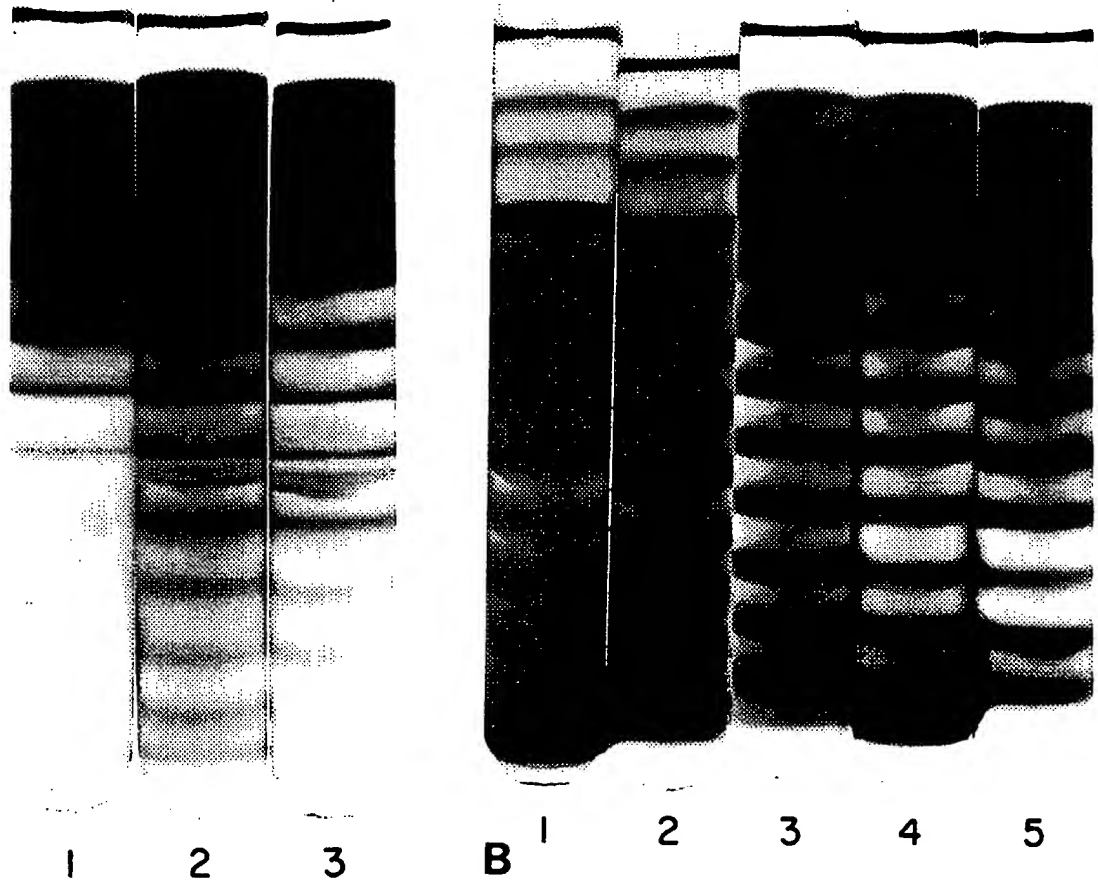

Fig. 2. Effect of hydroxyurea (HU) on the density profile of erythrocytes on discontinuous Stractan density gradients. A: Density profile of normal control RBC (lane 1) and of sickle RBC obtained from patient S.C. on day 343 of therapy (lane 2) and F.T. on day 335 of therapy (lane 3) after treatment with HU. B: Density profile of sickle RBC obtained from patients not treated with HU. Lanes 1 and 2 are from patients with four α genes, and lanes 3–5 are from patients with three α genes.

four α genes. This indicates that the α gene number seems to have a modulating effect on the maximum MCV that could be attained secondary to treatment with HU. The MCHC decreased in both cases as a result of improved cellular hydration secondary to HU, which is discussed below.

The increase in MCV after HU does not seem to explain the improvement in RBC deformability by itself for three reasons. First, cell deformability showed significant improvement 2–3 months after treatment, when the MCV was still in the upper normal range (Table II). Second, Burns et al. [5] reported that HU increased Hb F and the MCV of RBC of six patients (four with chronic myelogenous leukemia, one with psoriasis, and one with Hb SC disease) taking this drug. The maximum DI of RBC was determined on only one of these patients, the one with psoriasis, and was found to be within the normal range despite an MCV value of 130 fl and Hb F of 4.3%. Third, we have studied two white patients with myeloproliferative disorders taking HU for more than 1 year: the first patient took 500 mg/day, had an MCV of 119 fl and Hb F <1.0%; the second patient took 1,500 mg/day, had an MCV of 115 fl and Hb F of 4.5%. The RBC deformability of both patients was within the normal range. These findings suggest that the effect of HU on cell deformability is due not simply to macrocytosis but to other factors, such as improving the hydration status of sickle RBC, as discussed below.

Although HU improved whole cell and membrane deformability, it had no effect on membrane stability. Chasis and Mohandas reported [27] that RBC membrane deformability and stability are two distinct membrane properties that are independently regulated by skeletal protein associations. Membrane stability depends on the association of the junctional proteins (spectrin, actin, and band 4.1) of the skeletal network. Polyamines, for example, increase the association of these proteins and hence increase membrane stability [28,29]. The fact that

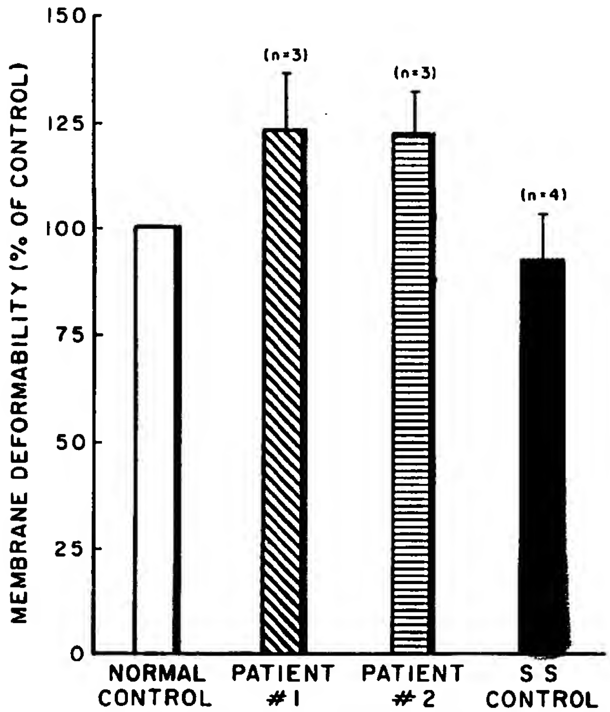

Fig. 3. Deformability of normal and sickle RBC membranes. Resealed ghosts were prepared as described in Materials and Methods, and deformability was measured at 100 dynes/cm² and expressed as a percentage of paired controls. Deformability of membranes from patient 1 is the mean ± SD of three determinations on days 337, 343, and 505 of treatment with HU and from patient 2 the mean ± SD of three determinations on days 335, 427, and 496 of treatment. Deformability of membranes from SS controls is the mean ± SD of four determinations on four different patients.

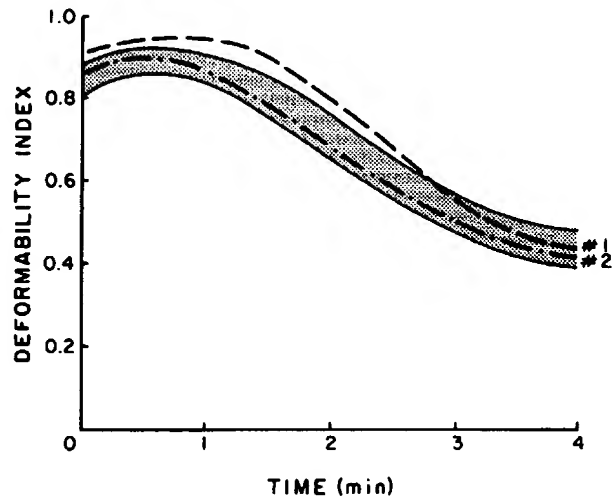

Fig. 4. Effect of hydroxyurea (HU) on membrane stability. This was determined on day 337 of treatment for patient 1 (SC) and on day 335 for patient 2 (FT). Resealed ghosts prepared from normal controls and from the patients treated with HU were exposed to high constant shear stress (750 dynes/cm²) in the ektacytometer and the decline of the deformability index (DI) was measured as a function of time. The rate of DI decline is a measure of membrane stability. In both patients, membrane stability was within the normal range (shaded area) after treatment with HU.

membrane stability remained normal after treatment with HU indicates that the rheological effects of this drug are not due to a megaloblastic effect. In megaloblastic anemia, RBC membranes have decreased stability [30].

Treatment with HU improved the hydration of sickle erythrocytes in both patients. This was shown by a decrease in the MCHC as measured with the H.1 system, increase in RBC K^+ concentration, decrease in percentage of dense RBC, and a shift in the Stractan cell density distribution toward less dense fractions and a right-shifted ektacytometric profile to high osmolarities [15,21]. These effects of HU are, undoubtedly, due mostly to its effect on Hb F production. Hb F inhibits the polymerization of sickle Hb and hence decreases the impact of its deleterious sequelae on RBC function, such as deformability, cation efflux, and cell density. Macrocytosis and improved intrinsic RBC membrane deformability, however, may be a unique property of the progenitors exposed to HU. One hypothesis suggests that these progenitors undergo premature erythroid differentiation to support reticulocyte production [31].

CONCLUSIONS

Together, the data indicate that, in addition to its effect on the production of Hb F, HU improves whole cell deformability, has no effect on membrane stability, decreases the percent of dense cells, and improves cellular hydration. Determination of the rheological properties of RBC may be of value in monitoring the response to HU used in the treatment of sickle cell anemia.

ACKNOWLEDGMENTS

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L35 ANSWER 2 OF 2 MEDLINE
AN 2001029686 MEDLINE
DN 20529031 PubMed ID: 11074924
TI Successful treatment of hepatitis C in sickle-cell
disease.
AU Swaim M W; Agarwal S; Rosse W F
SO ANNALS OF INTERNAL MEDICINE, (2000 Nov 7) 133 (9) 750-1.

L32 ANSWER 302 OF 324 MEDLINE
AN 89333692 MEDLINE
DN 89333692 PubMed ID: 2757007
TI Effect of hydroxyurea on the rheological properties of sickle
erythrocytes in vivo.
AU Ballas S K; Dover G J; Charache S
SO AMERICAN JOURNAL OF HEMATOLOGY, (1989 Oct) 32 (2) 104-11.

L41 ANSWER 4 OF 6 MEDLINE
AN 90205983 MEDLINE
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TI Hematologic responses of patients with sickle cell
disease to treatment with hydroxyurea.
AU Rodgers G P; Dover G J; Noguchi C T; Schechter A N; Nienhuis A W
NC HL-28028 (NHLBI)
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HEMATOLOGIC RESPONSES OF PATIENTS WITH SICKLE CELL DISEASE TO TREATMENT WITH HYDROXYUREA

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Abstract Because fetal hemoglobin contains gamma-globin chains instead of beta chains, it is not affected by the genetic defect that causes sickle cell disease. Increased levels of fetal hemoglobin decrease the tendency toward intracellular polymerization of sickle hemoglobin that characterizes this disease. Hydroxyurea is one of several cytostatic agents that have been shown to increase the production of fetal hemoglobin in some patients with sickle cell disease.

We studied the effects of hydroxyurea administration in 10 hospitalized patients with sickle cell disease, each of whom was treated for three months. Seven patients responded with a 2- to 10-fold increase in fetal hemoglobin, from a mean (±SD) of 1.6±1.6 percent of total hemoglobin to 6.8±4.7 percent; three patients had fetal-hemoglobin levels of 10 to 15 percent of total hemoglobin. Three did not respond to treatment. Four of the patients who responded were retreated with hydroxyurea after one to four months without treatment and were found to have larger increases in fetal-hemoglobin levels. In most patients, levels were still rising at the end of the study, even after 90 days of therapy. Fetal-hemoglobin levels tended to peak at dosages of hydroxyurea that were myelosuppressive. In the patients who responded to treatment, there were significant increases in the percentage of reticulocytes and erythrocytes containing fetal hemoglobin and in the amount of fetal hemoglobin within these cells. The percentage of dense red cells decreased in the patients who responded to treatment. The tendency toward intracellular polymerization at physiologic oxygen saturation was reduced by about 33 percent in the cells containing fetal hemoglobin, whereas there was no change in the other cells.

We conclude that hydroxyurea is effective in increasing the production of fetal hemoglobin, which in this study was found to be associated with a small decrease in hemolysis and an increase in hemoglobin levels despite myelosuppression. Controlled, prospective trials are necessary to establish whether these effects will lead to clinical benefit. (N Engl J Med 1990; 322:1037-45.)

THE finding that 5-azacytidine selectively increases the production of fetal hemoglobin in primates[1] led to clinical trials in which increases in fetal-hemoglobin levels were demonstrated in patients with beta-thalassemia[2] and with sickle cell disease.[3,4] Increases in fetal hemoglobin, resulting from increased gamma-chain synthesis, should be useful in the treatment of the beta-thalassemia syndromes by decreasing globin-chain imbalance[5] and in the treatment of the sickle cell syndromes because of the sparing effect of fetal hemoglobin on the polymerization of sickle hemoglobin.[6] Recently, other cytostatic agents, particularly hydroxyurea, have been shown to increase fetal-hemoglobin production in patients with sickle cell anemia.[7-11] Treatment of two severely affected patients with hydroxyurea, along with careful observations of several other patients treated for shorter periods, revealed a dramatic increase in the absolute fetal-hemoglobin level after several months of therapy. The total hemoglobin level also increased, reflecting a decrease in the rate of hemolysis.[10]

These studies have led to the consideration of prospective, clinical trials to establish the benefits of hydroxyurea in the treatment of sickle cell disease. For

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such trials to be feasible, more information is needed about the clinical pharmacologic features of hydroxyurea, including the optimal dosage regimens, and about predictive factors associated with maximal increases in the production of fetal hemoglobin. Recently, biophysical data on the inhibition of sickle hemoglobin polymer formation by fetal hemoglobin and epidemiologic data on the relation between fetal-hemoglobin levels and the clinical status of various patient groups have made possible estimates of the increases in fetal-hemoglobin levels that might be necessary for clinical benefit.[12] In this report we present data on 10 patients with sickle cell disease who were treated as inpatients for periods of three months (4 of the 10 patients were treated for an additional three-month period) in an attempt to clarify the pharmacodynamics of hydroxyurea, the increases in fetal hemoglobin and other hematologic responses to its administration, and the effects of such responses on the intracellular polymerization of sickle hemoglobin.

Methods

Patients with severe, longstanding complications of homozygous sickle cell disease, such as recurrent crises of severe pain, chronic bone pain, or severe symptomatic aseptic necrosis or intractable leg ulcerations, who had relatively well preserved renal and hepatic function were considered eligible for this study. The patients, eight men and two women, ranged in age from 22 to 42 years (average age, 37). The patients were hospitalized for approximately three months at the Clinical Center of the National Institutes of Health and were monitored carefully during the hydroxyurea trial. An exemption for treatment with an investigational new drug was obtained from the Food and Drug Administration. The treatment protocol was approved by the clinical research subpanel of the National Heart, Lung, and Blood Institute. After informed consent was obtained from the patients, we performed base-line and serial hematologic tests and measurements, including a complete blood count, reticulocyte count, hemoglobin electrophoresis, measurement of the fetal-hemoglobin level (as a percentage of total hemoglobin) by alkaline denaturation, profile of red-cell density,[13] and determination of the percentages of F reticulocytes and F cells.[14,15] Blood tests indicating the level of hepatic and renal function were performed three times a week; serum hydroxyurea levels one and four hours after the ingestion of the drug were measured once a week. Base-line serum erythropoietin levels were determined by radioimmunoassay (SmithKline BioScience Laboratories, Philadelphia). Restriction-endonuclease analysis of genomic leukocyte DNA was performed to assess the alpha-globin genotype[16] and the haplotype of the beta-globin gene cluster.[17]

Because previous studies indicated that diminished renal elimination of hydroxyurea could result in hematopoietic toxicity, the initial dose of hydroxyurea was based on duplicate determinations of hydroxyurea clearance.[9] The starting dose ranged from 10 to 20 mg per kilogram of body weight, taken orally in a single dose (Hydrea, Squibb, Princeton, N.J.) on four consecutive days each week. The dose was adjusted upward by increments of 5 mg per kilogram at four-week intervals until the patient reached an optimal dose or had had two dose adjustments. The dose was adjusted downward or maintained at a constant level if bone marrow suppression (defined as an absolute reticulocyte count <40,000 per cubic millimeter, a white-cell count <5000 per cubic millimeter, or a platelet count <150,000 per cubic millimeter) was observed. Patients were considered to be responding to treatment if they had more than a twofold increase in the percentage of F reticulocytes in total reticulocytes and the percentage of fetal hemoglobin in total hemoglobin. Four patients were retreated with what appeared to be the optimal dose of hydroxyurea for them after a period of one to four months without treatment. The retreatment dose was that at which increases in the fetal-hemoglobin level and the percentage of F reticulocytes occurred in the absence of a decline of more than 20 percent from the average of three base-line values in one or more peripheral-blood counts.

Statistical methods used to determine the significance of the differences in the paired sets of data included the paired Student's t-test for data with a gaussian distribution and the Mann–Whitney U test for data with a nongaussian distribution.[18] Univariate and multivariate analyses were used to determine the strength of relations between variables.[18] The period before a significant increase in the fetal-hemoglobin level and the F-reticulocyte level was termed the lag period. Lag periods were calculated from the intercept generated by fitting the initial and final data to two "best-fit" linear regression lines. The linear least-squares method[19] was used to determine the "best-fit" linear regression lines, and the final two lines were optimized for their respective correlation coefficients and residual errors.

The number of dense cells was defined as the percentage of cells with an intracellular hemoglobin concentration of more than 23.0 mmol per liter (37 g per deciliter), as measured by phthalate-ester gradient centrifugation.[13] The median corpuscular hemoglobin concentration (MCHC; the average hemoglobin concentration in erythrocytes),[10] which in normal persons is comparable to the mean corpuscular hemoglobin concentration, was determined from these gradients and used to calculate the tendency toward the intracellular polymerization of hemoglobin S for the bulk of the cells (i.e., the non-dense cells). Measurements of the mean corpuscular volume (MCV; the average volume of an erythrocyte) and the mean corpuscular hemoglobin (MCH; the average hemoglobin content of an erythrocyte) were obtained with use of a Coulter electronic cell analyzer. Calculation of intracellular polymerization was performed as previously described[12,20] on the basis of measurements of total intracellular hemoglobin concentration and the percentages of sickle hemoglobin and fetal hemoglobin. Although calculated for the full range of oxygen-saturation values, these measurements are presented here only for values at 70 percent oxygen saturation, the physiologically important range, for convenience of comparison. The polymerization tendency was calculated separately for the F cells and the non-F cells of the bulk of the cell population, as well as for the dense cells.

Results

Fetal-Hemoglobin Responses

Of the 10 patients treated for three months with increasing doses of hydroxyurea, 7 were considered to have responded to treatment because they had at least a twofold increase in the levels of F reticulocytes and fetal hemoglobin. Among those who responded, fetal-hemoglobin levels increased 2- to 10-fold, with three patients reaching maximal fetal-hemoglobin levels between 10 and 15 percent of total hemoglobin. Initial values for hemoglobin, reticulocytes, or fetal hemoglobin did not predict the response to treatment, since patients with a wide range of values for these indexes were included among both the patients who responded and those who did not (Table 1). Base-line values on tests of hepatic or renal chemistry, hydroxyurea clearance rates, and serum erythropoietin levels did not predict the ultimate response to treatment among the 10 patients (data not shown). We found no absolute correlation between either the alpha-globin genotype[16] or the specific beta-globin gene-cluster haplotype[17,21,22] and the production of fetal hemoglobin. In particular, the presence of the *Xmn*I restriction site[22] did not necessarily predict a favorable response to hy-

droxyurea treatment (Table 1). In the responding patients, there was a slight increase in total hemoglobin, despite the myelosuppressive nature of the doses of hydroxyurea. Although the moderate decline in the percentage of reticulocytes cannot be unequivocally interpreted, our finding of a decrease in the indirect bilirubin level (mean ±SD, 35.6±30.8 μmol per liter [2.1±1.8 mg per deciliter] before treatment and 18.8±15.4 μmol per liter [1.1±0.9 mg per deciliter] after treatment; $P<0.03$) in the patients who responded to treatment is consistent with a mild improvement in the hemolytic rate.

Table 1. Levels of Hemoglobin, Reticulocytes, and Fetal Hemoglobin before and after Treatment with Hydroxyurea and Presence or Absence of the *XmnI* Restriction Site in 10 Patients with Sickle Cell Disease.

Group and Patient	Hemoglobin (g/dl)*		Reticulocytes (%)		Fetal Hemoglobin (%)		*XmnI* Site†
	Initial	Final	Initial	Final	Initial	Final	
Group 1 (responders)‡							
A							=
First course	8.5	8.3	11.5	4.4	0.7	7.7	
Second course	8.3	9.2	16.3	9.2	2.6	9.7	
B	5.7	7.5§	15.6	3.2§	5.3	12.3	=
C							±
First course	11.2	10.2	4.9	5.1	0.9	3.6	
Second course	11.9	10.2	4.1	3.7	1.8	7.1	
G							±
First course	8.4	9.1	12.1	6.1	1.5	4.1	
Second course	8.7	9.9	6.6	4.9	5.4	7.3	
H							=
First course	6.1	7.0	15.3	8.7	1.3	13.9	
Second course	6.6	7.1	18.0	4.5	1.6	13.3	
I	7.6	8.9	6.9	5.4	0.4	1.4	=
J	8.6	8.5	3.4	3.0	1.5	4.5	=
First course (mean ±SD)	8.4±1.7	8.7±1.0§	9.0±4.6	5.4±1.9§	1.6±1.6	6.8±4.7	
Second course (mean ±SD)	8.9±2.2	9.1±1.4	11.2±6.9	5.6±2.5	2.8±1.8	9.4±2.9	
Group 2 (nonresponders)							
D	8.3	8.5	3.3	2.4	6.4	6.6	±
E	8.5	7.2	8.9	11.6	0.9	1.1	=
F	7.5	7.8	5.3	2.6	0.7	0.5	=
Mean ±SD	8.1±0.5	7.8±0.6	5.8±2.8	5.5±5.2	2.7±3.2	2.7±3.4	

*To convert values for hemoglobin to millimoles per liter, multiply by 0.6206.

†The symbol = denotes absence of, and ± heterozygosity for, the restriction-enzyme site.

‡Patients A, C, G, and H had a second three-month course of treatment at a fixed dose. Retreatment data are not included in the initial and final mean values but are averaged separately (second course).

§Patient B received two units of packed cells two weeks before his last hematologic measurements, at which time the percentage of hemoglobin A had increased to 30 percent. Values for Patient B are therefore not included in the calculations of the initial and final mean values for hemoglobin and reticulocytes.

Three factors determine the level of fetal hemoglobin in patients with sickle cell anemia: F-cell production, as measured by the percentage of F reticulocytes in total reticulocytes, the quantity of fetal hemoglobin per F cell, and the preferential survival of F cells (the ratio of the percentage of F cells to the percentage of F reticulocytes) as compared with red cells that lack fetal hemoglobin (non-F cells).[14,23] We measured these three variables in order to determine the basis for the increased fetal-hemoglobin level in the patients treated with hydroxyurea. As shown in Table 2, the increase in fetal hemoglobin observed in the patients who responded (Group 1) resulted primarily from an augmentation of F-cell production; F reticulocytes increased an average of sixfold, which accounted for about 70 percent of the increase in fetal hemoglobin. The quantity of fetal hemoglobin per F cell also increased in the responding patients during treatment, albeit more modestly, as did the percentage of F cells among total red cells. No effects of hydroxyurea administration on these three variables were observed in the patients who did not respond to treatment (Group 2). The initial values for the percentage of F reticulocytes, the percentage of F cells, the preferential survival of F cells, and the quantity of fetal hemoglobin per F cell did not predict the response to treatment, as patients who responded had values at both extremes for these indexes.

Figure 1 shows the pattern of fetal-hemoglobin and F-reticulocyte response in six responding patients during their initial course of therapy. Patient I (not shown) had values similar to those of Patient J, with respect to both base-line hematologic measures and the magnitude of the fetal-hemoglobin response to hydroxyurea. Among the responding patients, there was a spectrum of responses, evident in both the rapidity and the absolute magnitude of the change in the levels of fetal hemoglobin and F reticulocytes. Some patients (A, B, G, and I) had increased production of fetal hemoglobin after a prolonged lag period (average, 40 days), perhaps because the initial dose of hydroxyurea was suboptimal. Other patients (C and H) had increases in fetal hemoglobin within the first 20 days of treatment with hydroxyurea. In both groups of responders, the increase in fetal-hemoglobin levels was preceded and paralleled by an increase in the F-reticulocyte count. Patient J had a prompt increase in the F-reticulocyte count within two weeks of the beginning of treatment with hydroxyurea, yet it took an additional five to six weeks before increases in fetal hemoglobin were observed.

Retreatment

Of the seven patients who responded to the initial course of therapy, four were retreated with a second course of hydroxyurea at a fixed dose. This was the dose at which the levels of F reticulocytes and fetal hemoglobin rose, in the absence of hematologic toxicity (see Methods). Patients A and H received 20 mg per kilogram, Patient G received 25 mg per kilogram, and Patient C 30 mg per kilogram each day for four consecutive days per week. These patients were hospitalized for the second three-month course of treatment

Table 2. F-Reticulocyte Levels, F-Cell Levels, and Amount of Fetal Hemoglobin per F Cell (Hb F/F cell) before and after Treatment with Hydroxyurea in 10 Patients with Sickle Cell Disease.

GROUP AND PATIENT	F RETICULOCYTES (%)		F CELLS (%)		Hb F/F CELL (%)	
	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL
Group 1 (responders)*						
A						
First course	1.7	22.7	8.3	55.8	8.4	13.8
Second course	1.3	23.9	34.4	56.7	7.6	17.1
B	3.3	38.7	37.9	60.7	14.0	20.3
C						
First course	3.3	12.7	12.3	34.2	7.3	10.5
Second course	1.0	11.9	14.3	53.7	12.6	13.2
G						
First course	5.3	14.5	27.3	34.4	5.5	11.9
Second course	7.9	19.6	36.7	54.7	14.2	13.3
H						
First course	0.1	17.5	15.5	65.0	8.4	21.4
Second course	4.2	28.0	16.9	75.3	7.7	17.7
I	2.2	8.7	8.5	16.5	4.7	8.5
J	5.2	12.3	14.0	29.9	10.7	15.1
First course (mean ±SD)	3.0±1.9	18.2±10.1	17.7±11.0	42.4±18.2	8.4±3.2	14.5±4.8
Second course (mean ±SD)	3.6±3.2	20.9±6.9	25.6±11.6	60.1±10.2	10.5±3.4	15.3±2.4
Group 2 (nonresponders)						
D	8.3	22.0	40.4	50.9	15.8	13.0
E	1.9	1.3	7.1	20.3	12.7	5.4
F	1.0	0.7	7.0	11.3	10.0	4.4
Mean ±SD	3.7±4.0	8.0±12.1	18.2±19.3	27.5±20.8	12.8±2.9	7.6±4.7

*Patients A, C, G, and H had a second three-month course of treatment at a fixed dose. Retreatment data are not included in the initial and final mean values but are averaged separately (second course).

after an average of 100 days without treatment (range, 32 to 125 days). As Figure 2 shows, the average fetal-hemoglobin level before the second course of hydroxyurea therapy was slightly higher than the corresponding value before the first course of hydroxyurea. After the reinstitution of hydroxyurea treatment, there was a delay in the response of two of these patients (Patient A, 20 days; Patient C, 18 days), with little or no increase in fetal hemoglobin and F reticulocytes. Patient G, who returned for the second course of treatment after only 32 days — the shortest interval among the retreated patients — had continued to have increases in the fetal-hemoglobin level during the time when no hydroxyurea was given and had an initial fetal-hemoglobin value of 6.2 percent, which was higher than his discharge value, at the beginning of the second course. Since by this time his F-reticulocyte level (as well as that of the other patients) had returned to base-line levels, this higher level of fetal hemoglobin reflected the preferential survival of hydroxyurea-induced F cells in sickle cell disease.[14,23] In the four retreated patients, the final levels of fetal hemoglobin and F reticulocytes averaged 9.4±2.9 percent and 20.8±6.9 percent, respectively, after retreatment.

Marrow Suppression

During the first course, when dosages were increased, only mild marrow toxicity was observed (10 to 15 percent reductions in one or more counts) in the patients who did not respond to hydroxyurea (Group 2). On the other hand, among the patients who did respond (Group 1), there was a statistically significant decline in the reticulocyte count in four f seven patients (Table 1), in the white-cell count in five of seven patients, and in the platelet count in two of seven patients. However, the absolute nadir of these counts in all instances remained within the normal range for these hematologic values and therefore did not necessitate adjustments in the dosage. Despite the decrease in the reticulocyte count, most of the responding patients had some increase in total hemoglobin (Table 1), presumably because of the preferential survival of F cells and because of reduced hemolysis.

Although the four patients who underwent retreatment with hydroxyurea received a calculated "optimal" dose, each had evidence of hematopoietic suppression. The average magnitude of bone marrow depression observed was 28.3 percent for white cells ($P<0.04$) and 18.6 percent for platelets ($P<0.02$). Although the average decline in the reticulocyte count was 49 percent, because of the wide variations in the values among the four patients, this decline was not statistically significant, and dose reduction was not required. Thus, it may be concluded that doses of hydroxyurea that achieve fetal-hemoglobin responses are at or near the threshold of marrow suppression.

In most patients who responded during the first course of therapy and in the four who were retreated, levels of fetal hemoglobin were still increasing at the end of the three months. These data are consistent with the findings of Charache et al.[10] At the end of three months, three of the four patients on fixed doses of hydroxyurea reached a plateau in F-reticulocyte levels, suggesting the contribution of the preferential survival of F cells,[14] an increase in the amount of fetal hemoglobin per F cell, or both to the elevation in fetal-hemoglobin levels.

Hematologic Responses

The responding patients had striking changes in the MCV and MCH (Table 3). Among the seven responders, the MCV increased from 90.0±16.3 fl before treatment to 104.4±19.8 fl after three months of treatment with hydroxyurea ($P<0.006$). Similarly, there was an increase in the MCH from 30.0±5.8 to 34.7±7.2 pg per red cell ($P<0.02$). There was no net change in the MCHC in the responders. Using phthalate-ester gradients to monitor distributions of red-cell density,[13] we found that the patients who responded had a significant decrease

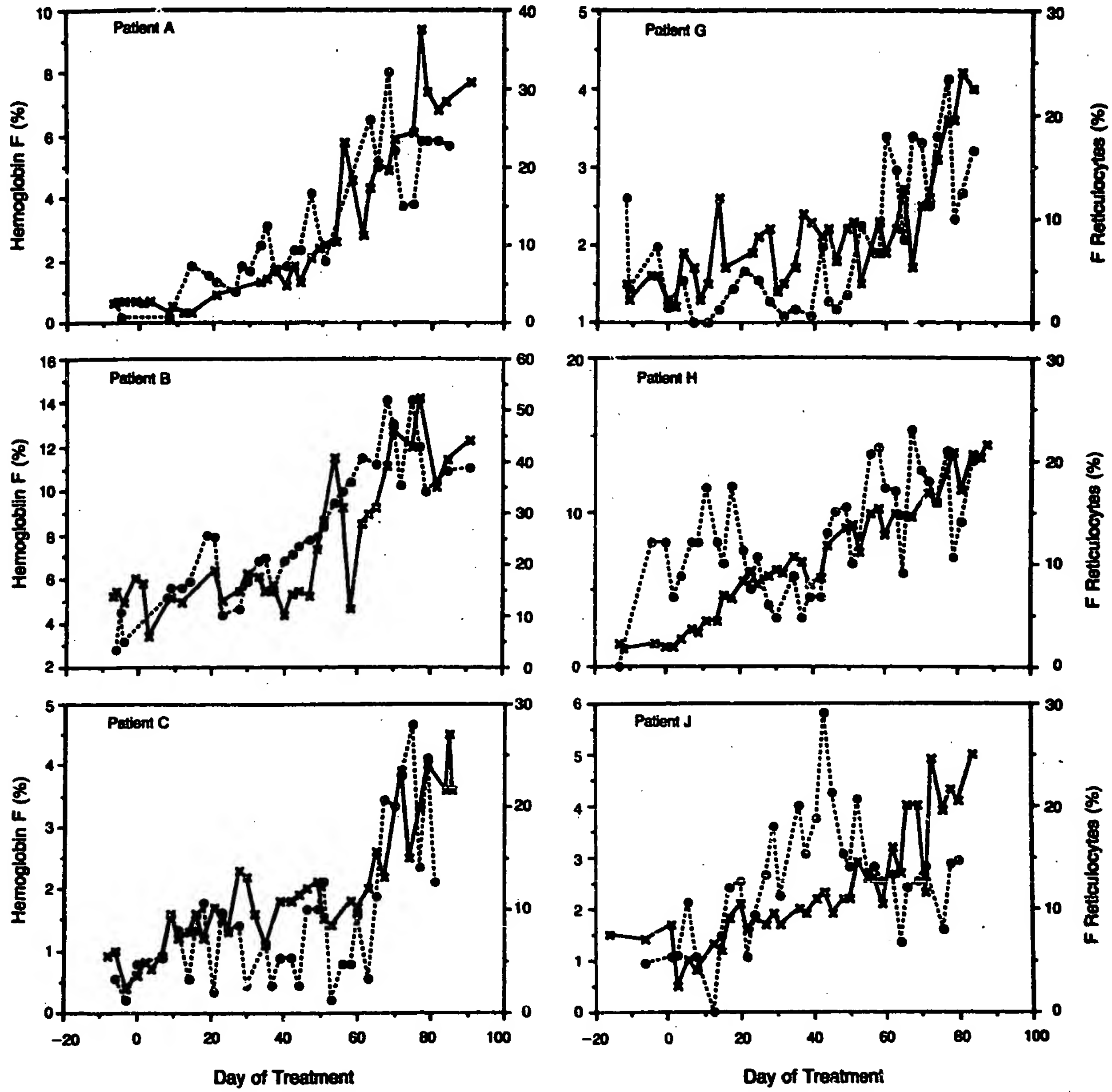

Figure 1. The Fetal-Hemoglobin (X) and F-Reticulocyte (●) Response in Six Patients Who Had Increased Fetal-Hemoglobin Production in Response to Treatment with Escalating Doses of Hydroxyurea over a Period of Three Months.

Patients C, G, H, I (not shown), and J received 15 mg, 20 mg, and 25 mg per kilogram for 30 days each, starting on day 0, day 30, and day 60, respectively. Patient A received 10 mg, 15 mg, and 20 mg per kilogram and Patient B received 20 mg, 25 mg, and 30 mg per kilogram beginning on day 0, day 30, and day 60, respectively. The increase in fetal hemoglobin observed in typical responders was associated with an increase in F-cell production, preceded and paralleled by an increase in the F-reticulocyte count.

in the percentage of dense cells (from 5.5±5.7 to 1.5±2.3 percent; P<0.01) and a slight decrease in the extent of cellular heterogeneity in corpuscular hemoglobin concentrations (R60 values [middle 60 percent density range]; from 0.014±0.004 to 0.010±0.004; P<0.01). There was no net change in the MCHC as measured by phthalate-ester gradients, a more sensitive indicator of the MCHC in patients with sickle cell disease.[13] In contrast, among the three patients who did not respond to treatment, only Patient E had changes in the red-cell indexes similar to those observed in the responders (Table 3).

Intracellular Polymerization

We examined the effects of hydroxyurea therapy on the tendency toward intracellular polymerization of sickle hemoglobin[12,20] in the responders. Since the beneficial hematologic effect of hydroxyurea in patients with sickle cell disease would be expected to

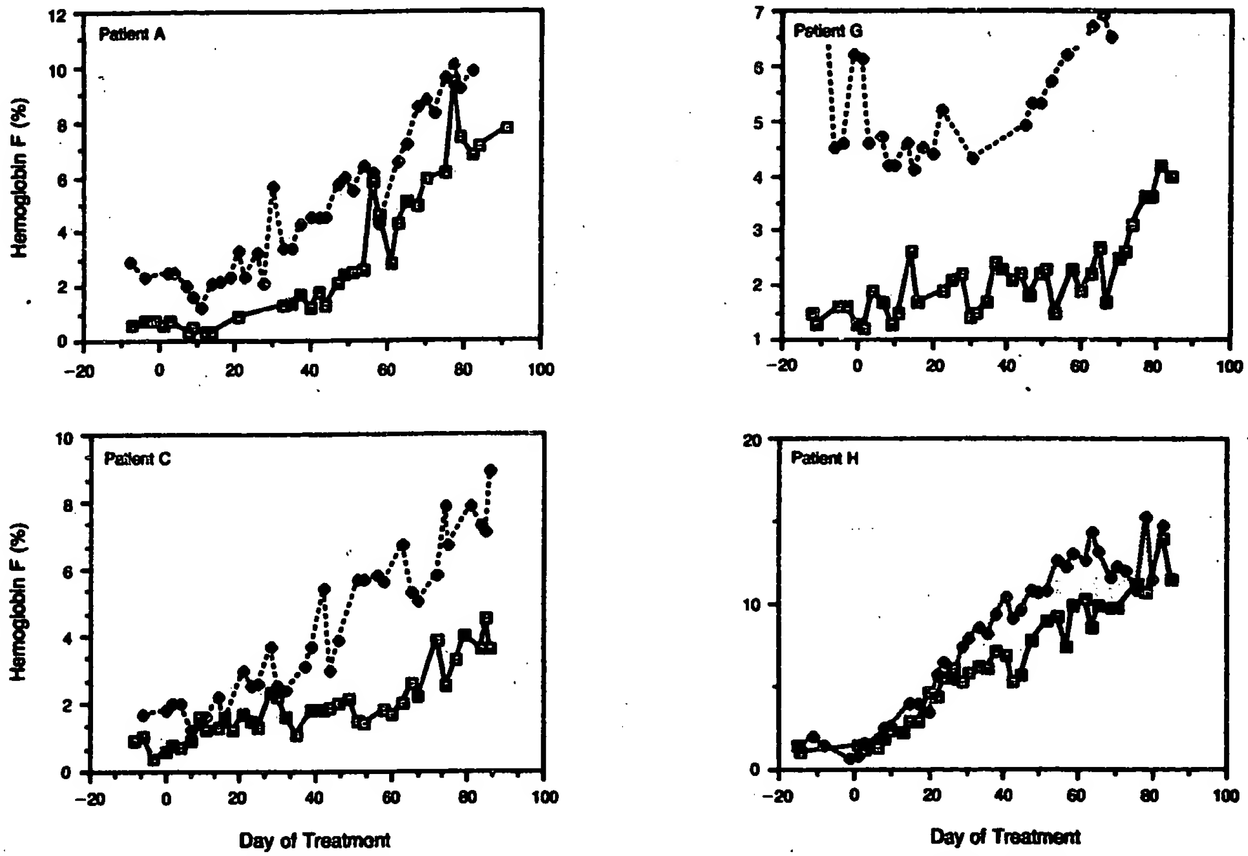

Figure 2. The Fetal-Hemoglobin Response in Four Patients to the Treatment with Escalating Doses (First Course [□]) and Fixed, "Optimal" Doses (Second Course [◆]) of Hydroxyurea.

During the second course, Patients A, C, and possibly G had lag periods before response, although this delay was significantly shorter than that observed during the first course.

be restricted to the F cells, the relative changes in the expected polymer fraction in F cells and non-F cells was considered. We calculated that there was a 33 percent reduction in the expected polymer fraction within F cells (from 0.09 to 0.06) at, for example, 70 percent oxygen saturation, as a result of the increased amount of fetal hemoglobin per F cell. The tendency to polymerization, as expected, remained unchanged (at 0.12) after hydroxyurea treatment in the bulk (non-F cell) population. Taking into account the change in the number of F cells after hydroxyurea therapy, as well as that in the number of dense cells, there was an average overall decline of approximately 25 percent (from 0.12 to 0.09) in the expected polymer fraction weighted for changes in the numbers of dense cells and F cells. This effect was seen in both the initial and the second course of treatment.

Discussion

Hydroxyurea has previously been shown to induce the synthesis of fetal hemoglobin in anemic primates[24,25] used as models of hematopoietic-response patterns and in patients with sickle cell disease,[7-11] by an unknown mechanism. One of the aims of this study was to identify predictive factors in patients with sickle cell disease that might be associated with maximal fetal-hemoglobin production. Previous trials of hydroxyurea in sickle cell disease have employed a starting dosage of 50 mg per kilogram,[7-9] a dose that leads uniformly to substantial hematopoietic suppression.[9,10] Accordingly, in this investigation, we used lower initial doses of hydroxyurea (usually 10 to 15 mg per kilogram), then gradually increased them by 5 mg per kilogram per month, in order to estimate a retreatment dose for maximal increases in fetal hemoglobin with minimal toxicity.

The three patients who did not respond to hydroxyurea had only minor signs of hematopoietic toxicity, both after the initial dose and after two increases in the dose. In contrast, of the seven patients who responded, two had a statistically significant decline in the white-cell count during the first month of treatment. Moreover, after the dose was increased, all the responders had some degree of peripheral myelosuppression. Thus, in our patients, therapeutic benefit was observed in most patients at a dosage of hydroxyurea that was myelosuppressive. This observation is consistent with the hypothesis that hydroxyurea may stimulate the production of fetal hemoglobin by sec-

Table 3. Values for Red-Cell Indexes before and after Treatment with Hydroxyurea in 10 Patients with Sickle Cell Disease.*

Group and Patient	MCH (pg)		MCV (fl)		MCHC (g/dl)		Dense Cells (%)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Group 1 (responders)†								
A								
First course	32.0	42.5	96.0	126.0	30.8	31.2	0.0	0.0
Second course	34.6	44.1	104.0	129.0	30.8	31.9	3.4	2.2
B	37.6	38.3	107.0	113.0	31.8	32.0	0.0	1.3
C‡								
First course	23.2	27.5	71.0	[illegible]4.0	32.8	31.9	8.5	0.0
Second course	22.6	28.8	70.0	89.0	33.0	32.6	7.0	2.5
G								
First course	28.5	32.3	88.0	99.0	33.0	33.0	2.1	0.0
Second course	30.3	33.0	92.0	101.0	33.1	33.0	3.2	0.0
H								
First course	36.4	45.0	112.0	133.0	31.2	31.3	2.7	0.0
Second course	36.1	46.8	104.0	133.0	32.4	32.0	4.1	1.8
I	29.5	29.4	86.0	91.0	32.3	32.1	14.2	5.8
J‡	22.8	27.8	70.0	85.0	31.6	31.9	10.8	3.6
First course (mean ±SD)	30.0±5.8	34.7±7.2	90.0±16.3	104.4±19.8	31.9±0.8	31.9±0.6	5.5±5.7	1.5±2.3
Second course (mean ±SD)	30.9±6.1	38.2±8.6	92.5±16.0	113.0±21.4	32.3±1.1	32.4±0.5	4.4±1.8	1.6±1.1
Group 2 (nonresponders)								
D‡	24.7	26.2	76.0	80.0	31.5	32.7	1.6	1.0
E	29.1	35.5	85.0	104.0	31.2	32.6	2.8	8.8
F§	33.1	24.3	96.0	75.0	31.5	31.7	3.5	1.3
Mean ±SD	29.0±4.2	28.7±6.0	85.7±10.0	86.3±15.5	31.4±0.2	32.3±0.6	2.6±1.0	3.7±4.4

*MCH denotes mean corpuscular hemoglobin, MCV mean corpuscular volume, and MCHC median corpuscular hemoglobin concentration.

†Patients A, C, G, and H had a second three-month course of treatment at a fixed dose. Retreatment data are not included in the initial and final mean values but are averaged separately (second course).

‡Patients C and J had homozygous alpha-thalassemia (−α/−α); Patient D had heterozygous alpha-thalassemia (−α/αα). All the other patients had the normal four alpha-globin genes.

§Patient F, who had four alpha-globin genes and a history of iron overload, had values indicating microcytosis and hypochromia with continued elevations in serum iron and ferritin levels during treatment with hydroxyurea.

ondarily inducing erythroid regeneration.[8] On the ther hand, recent in vitro studies of erythroid progenitors from patients with sickle cell disease who had been treated with hydroxyurea suggest that other mechanisms may also be at work.[26] In our study, no patient required either adjustments in the dosage or discontinuation of treatment because of clinical complications. This suggests that the patients who did not respond might tolerate higher doses of hydroxyurea than were given during the 90-day trial period; if the doses were increased to levels equal to or near those causing marrow suppression, fetal-hemoglobin production might increase in these patients, as was observed in the seven patients who responded to treatment.

There were no correlations between the initial hemoglobin level, reticulocyte count, fetal-hemoglobin level, F-reticulocyte level, amount of fetal hemoglobin per F cell, serum erythropoietin and serum hydroxyurea levels, or the results of blood tests for renal or hepatic function and the patient's subsequent response to hydroxyurea. Moreover, we were unable to detect a relation between the alpha-globin genotype[16] or the DNA polymorphisms, such as the *Xmn*I restriction site within the beta-globin gene cluster,[17,21,22] and the F-cell response.

Three of the responders and one of the nonresponders had been treated previously with 5-azacytidine and had had similar responses[27]; this fact raises the possibility that there exists an as yet unrecognized genetic determinant in patients for F-cell responsiveness, which is activated by both drugs. The constant presence of macrocytosis in our patients (Table 3) and in those in other studies[10,28,29] who responded to hydroxyurea by increasing fetal-hemoglobin synthesis may indicate that factors controlling F-cell production may interact directly or indirectly with the determinants of erythroid-volume regulation.

The patients who responded to hydroxyurea were notably heterogeneous with respect to the pattern and rapidity of their response. The range of the increase in fetal hemoglobin was 2- to 10-fold; three patients had levels between 10 and 15 percent of total hemoglobin during the three-month trial. Using multivariate analysis to dissociate the contributions of the three factors that determine levels of fetal hemoglobin in patients with sickle cell disease,[14] we found that F-cell production, as estimated by F-reticulocyte levels, accounted for about 70 percent of the increase in fetal hemoglobin in these patients, with a smaller contribution resulting from an increase in the quantity of fetal hemoglobin per F cell. The preferential survival of F cells,[14,23] presumably the result of decreased intracellular polymerization of hemoglobin S, became a more prominent factor in the patients retreated after an interval during which hydroxyurea was withheld. With long-term hydroxyurea therapy, fetal-hemoglobin levels may contribute a larger proportion t the final steady-state hemoglobin value. Alternatively, as evidenced by the continued increase in the level f fetal hemoglobin in Patient G between the first and the second course of treatment (Fig. 2), intermittent-treatment strategies may be developed to exploit the preferential survival of F cells in these patients.

There was a lag period between the initiation of treatment with hydroxyurea and the subsequent increases in fetal hemoglobin (or F reticulocytes) in two of the four patients who underwent retreatment. These results, which were obtained when the patients were hospitalized and closely monitored, indicate that hydroxyurea should be given for a trial period of at least 60 days before a patient is determined not to be responding to the drug.

The calculated steady-state polymer fraction at 70 percent oxygen saturation at the median cell density, which may be considered a rough guide to the efficacy of treatment, was unchanged in the non-F cells but declined by about 33 percent, to an average polymer fraction of 0.06, in the F cells. This is still a substantial polymerization potential,[12] slightly higher than in cells from a patient with the mild sickle cell syndrome sickle hemoglobin-${}^{G}\gamma^{A}\gamma$-β^{0}-HPFH (0.05 at 70 percent oxygen saturation), for example.[12,30] There remain many non–F-containing cells in treated patients, which would retain their intrinsic tendency to form intracellular polymers under physiologic conditions and thus would be expected to continue to manifest their hemolytic and vaso-occlusive propensities. However, a second mechanism of possible benefit is the decrease in the number of dense cells. Although this effect was noted in an initial study of patients with sickle cell disease who were treated with 5-azacytidine,[3] it has been an inconsistent finding in other studies.[10] The overall decrease in dense cells, from 5.5 to 1.5 percent in our study, would also contribute to lowering the overall tendency toward the intracellular polymerization of hemoglobin S.[16,20] A "weighted" polymer fraction was calculated on the basis of this decrease as well as the increase in the F-cell fraction and the amount of fetal hemoglobin per F cell. The analysis showed a decline of about 25 percent in the overall polymer fraction after treatment, from 0.12 to 0.09.

It is possible that further reductions in the polymer fraction might be achieved through other hydroxyurea regimens. In many of the patients who responded, fetal-hemoglobin levels were still rising at the end of the 90-day protocol. Longer periods of therapy might achieve higher steady-state levels of fetal hemoglobin. Daily hydroxyurea treatment might also result in higher fetal-hemoglobin levels[28,31] than intermittent therapy. The increase in F cells in baboons treated with recombinant human erythropoietin is also of great interest in this regard.[32] The study by Al-Khatti et al.[32] and that by McDonagh et al.[33] suggest that erythropoietin may act synergistically with hydroxyurea to increase both the number of F cells and the level of fetal hemoglobin. Therefore, treating patients with hydroxyurea for longer periods of time, with alternative dosage schedules, or with hydroxyurea in conjunction with agents such as erythropoietin may lead to higher levels of fetal hemoglobin and greater inhibition of the polymerization of sickle hemoglobin.

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INDICATORS OF PROGNOSIS IN NODE-NEGATIVE BREAST CANCER

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Abstract Measures of the proliferative activity of tumor cells have prognostic value in patients with node-negative breast cancer. We studied 367 women in southern Sweden who had undergone surgical resection for such cancer. Tumor specimens were analyzed with DNA flow cytometry in order to estimate both the DNA content (ploidy) and the fraction of cells in the synthetic phase of the cell cycle (S phase). The median duration of follow-up was four years; 28 percent of the patients received adjuvant therapy, usually with tamoxifen (n = 83).

A multivariate analysis based on complete data on 250 patients included the following covariates: age (≥75, 50 to 74, and ≤49 years), tumor size (≤20 vs. >20 mm), concentration of estrogen and progesterone receptors (<10 vs. ≥10 fmol per milligram of protein), ploidy (diploid vs. nondiploid), and S-phase category (fraction of cells in S phase: <7.0 percent, 7.0 to 11.9 percent, and ≥12 percent). The S-phase fraction yielded the most prognostic information, followed by progesterone-receptor status and tumor size. A prognostic model based on these three variables identified 37 percent of the patients as constituting a high-risk group with a fourfold increased risk of distant recurrence. In the remaining 63 percent of the patients, the five-year overall survival rate (92±4 [±SE] percent) did not differ from the expected age-adjusted rate for Swedish women.

We conclude that a prognostic index that includes indicators of the proliferative activity of tumor cells may be able to identify women with node-negative breast cancer in whom the risk of recurrence is sufficiently low that adjuvant chemotherapy can be avoided. (N Engl J Med 1990; 322:1045-53.)

LONG-TERM follow-up studies of survival show that in most cases, primary breast cancer is a systemic disease that may recur decades after it has been initially diagnosed.[1] However, findings from studies of the survival of patients with this disorder suggest that the disease takes two forms: one that is rapidly fatal, and another in which the outcome differs little from that of women of similar age without disease.[2]

Most women with node-negative breast cancer who receive only local treatment will survive without further symptoms and eventually die of other causes.[1,3] The proportion of such women is probably increasing as screening programs allow breast cancer to be diagnosed at an earlier stage. In the future, more than half of women with newly diagnosed breast cancer will have node-negative disease.[4]

Both the proliferative activity of tumor cells as assessed with the thymidine-labeling technique and the DNA content of individual tumor cells as determined by static (image) cytometry have been shown to yield prognostic information in node-negative disease.[5-9] Flow-cytometric measurements of DNA provide information about both DNA ploidy and proliferative activity as represented by the fraction of cells in the DNA synthetic phase, or S phase, of the cell cycle. In a recent report Clark et al.[10] evaluated the prognostic value of the combination of data on ploidy and data on the S-phase fraction in cases of node-negative disease; after these investigators had adjusted for other prognostic factors, they found that ploidy was an independent prognostic factor and the S-phase fraction yielded additional prognostic information only if the tumor was diploid.

On the basis of results from randomized clinical trials, it has been recommended that some sort of systemic adjuvant therapy should be given to all women with node-negative breast cancer[11,12]; however, if all

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